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PART I. THE RELATION OF INSECT RESPIRATION IN GRAIN TO HEAT AND MOISTURE PRODUCTION

DAVID L. LINDGREN[1]

INTRODUCTION

Heating and insect infestation are the two most important types of damage to which stored grains are subject. The former is followed by decomposition, whereas the latter may or may not be followed by heating and decomposition. It has been known for centuries that grain will heat when stored in large amounts in a damp condition, but the cause was not determined until rather recently. Bailey and Gurjar (1918) state: "That the phenomenon known as respiration is responsible for the heat energy released in a mass of damp grain is shown by modern research." These authors present a historical summary of the various theories that have been set forth concerning the causes of heating of vegetable matter, and also the factors which affect the rate of respiration in grain.

Respiration in its widest meaning is sometimes defined as the gaseous exchange between an organism and its environment, but strictly speaking it is a part of metabolism, because it marks the beginning and the end of the oxidation process. In aerobic respiration the oxygen of the air diffuses or is conducted to the cells where oxidation occurs, the end products being carbon dioxide, water, and heat. Respiration is an exothermic reaction, and can best be shown by the following equation:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 677.2 \text{ callories}$$

The carbon dioxide diffuses or is conducted from the cells to the atmosphere. This equation shows that the amount of carbon dioxide expired bears a definite relation to the quantity of heat energy liberated.

It is generally known that the heating of grain is sometimes initiated by insects, and their development makes it possible for other agencies to carry on the destruction. Back and Cotton (1924) say the most prevalent opinion is that heating is due to fermentation started by the attraction of moisture to the feces, but they do not believe this to be the probable cause. Doran (1893) expressed the opinion that heating of grain was due to the conversion of a certain amount of so-called

[1] The writer wishes to express his appreciation to Dr. H. H. Shepard, under whose direction the work was done, for his assistance and encouragement, and to Dr. C. H. Bailey for his stimulating interest in the problem.

"latent heat" into sensible heat at the time of the metamorphosis of the insect. Some persons have believed the heating to be caused by the friction of the insect mouthparts against the grain during feeding.

It seems that the chief factors causing insect-infested grain to heat have been overlooked; i.e., the production of heat and metabolic water as end products of insect respiration. The heat produced by the insects probably plays no small part in raising the temperature of the grain, and a certain amount of the metabolic water produced by the insects is absorbed by the grain with a resulting higher moisture content of the grain. This rise of both the temperature and the moisture content of the grain may or may not be of importance in itself, but it brings about conditions which are very favorable to the rapid increase of respiration in the grain. Bailey and Gurjar (1918) show that biological oxidation of wheat through the action of oxidizing enzymes is accelerated by an increase in temperature and moisture. With these factors working together, a rapid destruction and decomposition of the grain takes place. As Bailey and Gurjar (1918) and Bailey (1921) have thoroly covered the field of respiration of wheat and corn, this bulletin will be confined to the part the insect contributes.

HISTORICAL SUMMARY

Duhamel du Monceau and Tillet (1762) observed the heating of grain by insects. They say, according to Flanders (1933), "Is it produced by the heat of the insects themselves which are found assembled in great numbers? That could be because it is known that there is a considerable degree of heat in hives that are well populated by bees."

L. O. Howard (1888) cited a case of *Bruchus scutellaris* causing a rise of 25° C. in a sack of cowpeas. He believed the difference in temperature was evidently due in great part to a mechanical cause, the gnawing of peas by the beetles and larvae.

Doran (1893) observed a rise of 53° F. in a bin of perfectly dry middlings which was badly infested with the common red beetle *(Silvanus cassiae)*. He ruled out mechanical friction as a cause of this rise and concluded that during the early part of metamorphosis latent heat is changed into sensible heat or a certain amount of heat energy is expended in the operation.

In the classical work of Babcock (1912) on metabolic water he states: "The energy required for maintaining the vital functions of animal cells is derived from the oxidation of nutrients, through the respiration of protoplasm. The nutrients are oxidized within the cell wall, where all of the resulting products are set free. These products, which consist principally of carbon dioxide and water, are gradually

removed from the cell by osmosis and diffusion." In other words, insects should be able to raise both the temperature and moisture content of a quantity of grain through their metabolic activities. Robinson (1926) has shown that grain weevils are able to initiate a rise in moisture content of the wheat, altho the surrounding atmosphere was very dry. Back and Cotton (1924) concluded from their observations that the insects themselves are capable of developing heat in grain in storage.

Baston, as cited by Back and Cotton (1924), found insect-infested wheat heating with a moisture content as low as 11 to 12 per cent. He also found wheat free from insect infestation to have a higher moisture content than infested grain, contrary to what would be expected.

According to Chapman (1928), it is not strictly true that the body temperature of poikilothermic animals is always the same as the temperature of the surrounding medium. During the time of activity a certain amount of energy is continually being transformed into heat in the body; and as long as the rate of radiation from the body is less than the rate of production of heat within, it follows that the body temperature will be somewhat above that of the surrounding medium. The heat of infestation of grain may therefore be partly due to the direct transference of the body heat of the insect to the grain.

Flanders (1933) found that the heat given off by insect populations of *Sitotroga cerealella* increased as the larvae increased in size, until most of them had become full grown and had spun their cocoons. From the time the first moth emerged, the heat decreased until emergence was complete, then the heat of infestation disappeared and the temperature returned to that of the normal uninfested jars. Takahashi (1931 and 1933) states that very little attention has been paid to the important problem of the heating of the stored grains as a result of infestations by such insects as *Calandra* sp., *Bruchus* sp., *Sitotroga cerealella,* etc. He believes that the heat is produced by the larvae, but not by the adults, and seems to have no connection with the fungi growing on the excreta or injured grain. He states: "Heating of stored grain is not caused by the adults of *Calandra oryzae* but only by the larvae, and is highest when the larvae are 12 days old. This is the period at which the respiration and the temperature of larvae are the highest." Livingston (1934) states: "That the respiratory process produces water as well as carbon dioxide has long been known, but the possible importance of water so derived has received but little attention." He calculates the metabolic water produced by young wheat seedlings by measuring the carbon dioxide output at various temperatures and oxygen combinations. He concludes his paper with the following statement: "Direct measurement of rates of production of

respiration water appears to be difficult in the present stage of physiological technique, but measurements may be made of rates of volume increase, dry weight change, carbon dioxide production, and oxygen absorption."

MATERIALS AND METHODS OF STUDY

It is now well known that the respiratory exchange of an organism is one of the best criteria of metabolic activity. Measurements can be made of either the oxygen consumption or the carbon dioxide output, or both. Within recent years the methods for the determination of carbon dioxide have been greatly improved and simplified and can be easily adapted for work on insects. Therefore carbon dioxide output was used in the present study to obtain quantitative measurements of the metabolic processes taking place in three species of insects.

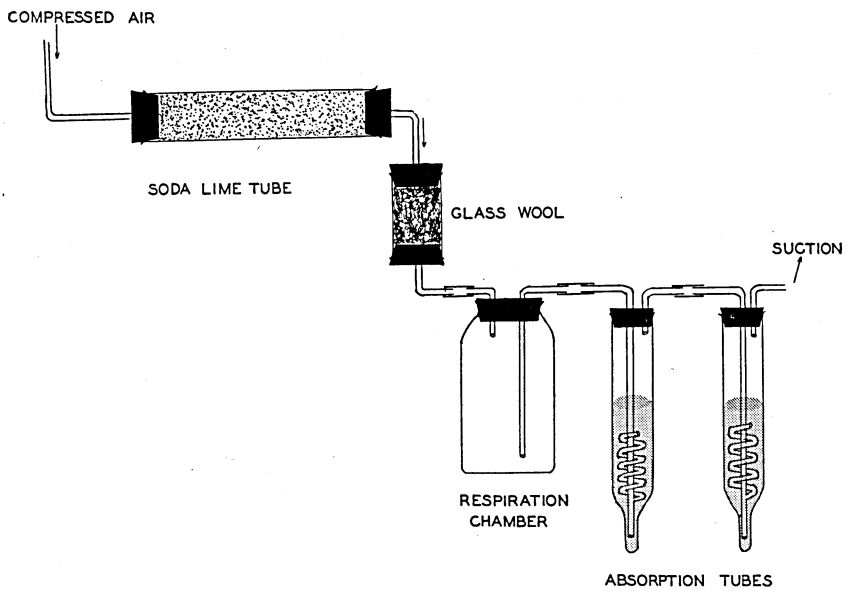

Fig. 1. Apparatus Used in Determining the Carbon Dioxide Output of Insects

The set-up for carbon dioxide determination is shown in Figure 1. It consists of a soda lime tube, glass wool container, respiration chamber, and absorption tubes. The latter are patterned after those used in the plant physiology laboratories at University Farm, St. Paul. Compressed air is passed through the soda lime tube which removes all the atmospheric carbon dioxide, and then through the glass wool which removes any impurities such as particles of soda lime. The air stream

passes on to the respiration chamber, in which are located the insects and their food supply. The air comes in at the top of the jar and passes out through a capillary tube projecting to within one-half inch of the bottom. By this arrangement a maximum flushing of the respiration chamber is obtained. Capillary tubing was used between this chamber and the absorption tubes so as to have the smallest possible amount of air space between the two. The air mixing with the carbon dioxide of the respiration chamber (capacity, 950 cc.) passes to the absorption tubes which contain measured amounts of barium hydroxide. As the air and carbon dioxide are aspirated through the barium hydroxide solution, the carbon dioxide is precipitated as barium carbonate. This apparatus was checked to ascertain whether any carbon dioxide from the air was finding its way into the system. Measured quantities of barium hydroxide were placed in the absorption towers and carbon dioxide–free air drawn through for as long as six hours. The tubes were then removed and the solution titrated. The titrations disclosed no measurable quantities of carbon dioxide present. In order to test the efficiency of the absorption, definite quantities of carbon dioxide were evolved in the respiration chamber and drawn into the absorption tubes. In no case did the amount obtained vary from the theoretical by more than two per cent.

The standard hydrochloric acid solution used for titration and for standardizing the alkali solution was 0.204 normal. This was standardized originally against dry sodium carbonate as described by Olsen (1916, p. 264). The standardization was repeated from time to time to ascertain whether changes occurred in the acid solution.

In preparation of the standard alkali solution the directions given by Truog (1915) were followed. Forty grams of Baker's analyzed barium hydroxide were dissolved in 1,000 cc. of water, heated to boiling and then filtered through a Buchner funnel into a stock bottle free from carbon dioxide. About 16 liters of this solution was prepared and was kept in a large 20-liter bottle with a two-hole rubber stopper carrying a glass siphon for withdrawal of solution and an air inlet protected by a soda lime tube to exclude carbon dioxide. The small amount of barium carbonate present was allowed to settle before using the clear solution. That no carbon dioxide entered the system was shown by repeated tests of the solution as delivered by the burettes, titration being made with the standard acid solution and phenolphthalein.

The total amount of carbon dioxide precipitated during an interval was expressed in terms of the equivalent volume of standard acid solution, in cubic centimeters, and this value was then multiplied by the standard factor 4,488, as 1 cc. of hydrochloric acid solution had been

shown to be equivalent to 4.488 milligrams of carbon dioxide. The resulting product was the number of milligrams of carbon dioxide produced by the insects in a given time. This divided by the weight of the insects gave the weight of carbon dioxide per unit weight of insect.

A typical experiment was carried out in the following manner. One hundred rice weevils or 70 granary weevils were placed in a respiration chamber with a weighed amount of wheat. One hundred rice weevils weigh approximately as much as 70 granary weevils. The wheat had been brought up to the proper moisture content by adding water and allowing it to stand two weeks with occasional shakings before it was used. Wheat was used only between the second and fourth weeks after water had been added, as Bailey and Gurjar have shown that the period of dampness bears a relation to the rate of respiration. One hundred grams of wheat calculated on a dry basis were used in all the experiments. Moisture was determined by the Brown-Duval method. After the insects and the wheat were in the respiration chamber, carbon dioxide-free air was drawn through for three minutes before sealing. The jars were then placed in a constant temperature cabinet where they were kept for 46 hours before the respiration test was run. The respiration chamber was then hooked into the set-up, as shown in Figure 1, and air drawn through for two hours, making the total length of the experiment 48 hours. The titration was made directly in the absorption tubes. Forty-eight hours was chosen as the standard time after much experimentation with 24-, 48-, and 72-hour periods. Twenty-four hours seemed to be too short as the great activity of the insects after handling influenced the final results somewhat, whereas in the use of a 48-hour period this error would be divided by two. It was feared that 72 hours would be too long as there might be sufficient carbon dioxide present to depress the respiratory rate.

The wheat used was obtained from the Division of Agronomy of the University of Minnesota. It was of the Ceres variety, a hard red spring wheat. The moisture content when obtained was about 9 per cent. The kernels were rather small and shriveled, probably due to the drouth condition of the 1934 growing season.

The insects used were the adults of *Sitophilus oryzae* Linn. and *Sitophilus granarius* Linn. The temperature at which they were reared varied between 23° and 25° C. At first the moisture content of the wheat of the stock cultures was not controlled; that is, the wheat in a stock culture of the rice weevil may have contained 15 per cent moisture, while that of the granary weevil may have contained only 12 per cent. It was soon seen, however, that this factor had to be controlled for both cultures, as the weight of the individuals was very much affected even

tho perhaps the metabolic rate was not. By rearing the stock cultures of granary weevils in wheat of a low moisture content, their weight was so decreased that the figure representing their carbon dioxide output in terms of milligrams of carbon dioxide per gram insect could be so increased that their metabolic rate appeared to be much greater than that of the rice weevil. Therefore the wheat of both cultures was kept as close to 15 per cent moisture content as possible. The insects were counted and weighed before and after each experiment, the carbon dioxide output being calculated from the original weight.

All experiments were carried out in triplicate, both for insects and wheat, and for wheat alone. These were run as closely in succession as possible. The carbon dioxide produced by the wheat alone was subtracted from the carbon dioxide produced by the wheat and the insects together. This necessitated three experiments for wheat alone, three for the rice weevil, and three for the granary weevil at each moisture content and temperature. The average of the three experiments was taken as the carbon dioxide output for the series. In any one series of three experiments a variation of 15 per cent was occasionally obtained, but a difference of between 10 and 15 per cent was the usual case. As the respiratory exchange is an extremely variable quantity and depends so much on the functional activity, this discrepancy does not seem so large. These variations may be due to any one of several factors. Any movement of the respiration flasks during the experiment tended to excite the insects, so they would immediately come to the top of the wheat and crawl around actively. Even vibrations in the cabinet may disturb them. The amount of air drawn through the chamber at the end of the experiment was held constant at seven to eight liters for two hours, as the movement of air caused the insects to become active. Many experiments were conducted to determine the effect of light on the rate of metabolism, but the results were negative, the carbon dioxide output being practically the same in darkness as in light.

THE RELATION OF INSECT INFESTATION IN GRAIN TO CARBON DIOXIDE PRODUCTION

Carbon Dioxide Production of Wheat

As Bailey and Gurjar (1918) thoroly covered the field of respiration in stored wheat, only those experiments on wheat were carried out in the present work which were necessary to arrive at definite figures for the part the wheat contributed to the total carbon dioxide output of wheat and insects combined. These figures are given in Table 1. Each figure is the result of three or more experiments averaged together. The

figures are slightly higher than those Bailey gives for the respiration of Haynes Bluestem wheat at various moisture contents and temperatures, but the trend is very nearly the same. The higher readings received here are perhaps due to the smaller shriveled kernels, with relatively more embryo present per gram wheat. Evidence tends to show that the seat of oxidative activity is in the embryo. It is of interest to note that the rate of carbon dioxide production increases rapidly at a moisture content above 14 per cent. It is below this moisture content of wheat that there is the greatest weight loss and mortality among the insects.

Table 1.—Respiration of Wheat, Incubated for 48 Hours

Per cent moisture content	Carbon dioxide production per 24 hours for each 100 grams dry matter	
	25° C.	35° C.
8.7	trace	trace
10.7	trace	0.22
14.0	0.67	1.45
15.2	1.00	2.24
17.4	13.2	21.2

Carbon Dioxide Production of *Sitophilus oryzae* (the Rice Weevil)

To determine whether the moisture content of the wheat would affect the carbon dioxide output of the weevils, the following series of experiments was performed: Wheat was prepared at different moisture contents varying from 8.7 to 17.4 per cent and the respiration of the weevils tested at these various moisture contents. The data for the rice weevil are given in Table 2, the period of exposure being 48 hours. In Figure 2 the same results are shown but are divided by two to indicate the average for 24 hours.

Table 2.—Respiration of *S. oryzae* in Wheat of Various Moisture Contents

Per cent moisture content of wheat	Per cent loss in weight of weevils		Per cent of dead weevils		Mgs. CO_2 per gram of weevils	
	25° C.	35° C.	25° C.	35° C.	25° C.	35° C.
8.7	26.7	44.2	35.2	100.0	151.6	129.0
10.7	13.6	32.3	3.6	77.6	300.0	215.4
14.0	1.5	8.3	3.0	4.3	412.8	522.6
15.2	0.9	3.7	0.0	1.6	403.8	578.8
17.4	0.2	0.2	0.0	0.9	409.2	464.0

Below 14 per cent moisture content of the wheat, the loss in weight and mortality of the weevils are high. These figures are higher than those Robinson (1926) obtained. He found 27 out of 50 *S. oryzae* alive at the end of six days in wheat having a moisture content of 8.2 per cent. However, he does not mention the temperature at which his

experiments were carried out, and by comparing the loss of weight and mortality at 25° C. with those at 35° C. it is easily observed that temperature is a very important factor. For instance, at a moisture content of 10.7 per cent and 25° C., 3.6 per cent of the insects were dead at the end of 48 hours, while at 35° C. 77.6 per cent were dead. Barnes and Grove (1916) have shown that *S. oryzae* is very sensitive to dryness, and that at any given moisture condition the length of life decreased with an increase in temperature. In wheat having a moisture content of 14 per cent or higher the loss of weight and the mortality of the insects are very low, and in many cases there is no loss in weight and no mortality.

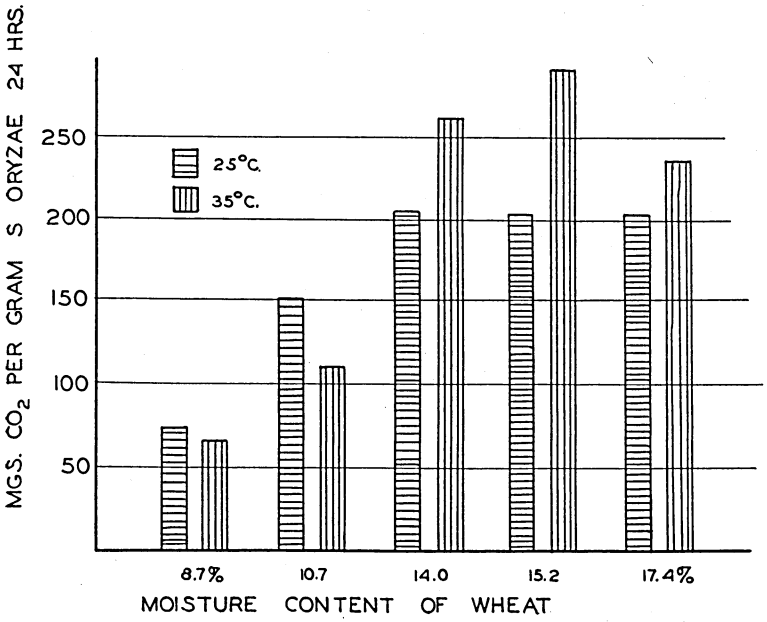

Fig. 2. Respiration of *S. oryzae* in Wheat of Various Moisture Contents

Below 14 per cent moisture content of wheat the carbon dioxide output of the weevils was greater at 25° C. than at 35° C., owing probably to the higher death rate at the latter temperature. At 14, 15.2, and 17.4 per cent and 25° C. the metabolic rate as measured by the carbon dioxide output is practically constant. This is well demonstrated in Figure 2. At 35° C. the carbon dioxide production by the insect reaches a peak at 15.2 per cent moisture content of the wheat and drops off considerably, progressing either way from this high point. The greater number of dead at a moisture content below 14 per cent

may account for part of this discrepancy but not for all of it. But a higher mortality means that unfavorable conditions existed for the remaining insects, these conditions being thereby reflected in a lower rate of metabolism. The same may be said about the low carbon dioxide output of the insects at 25° C. and 10.7 per cent in comparison with those at 25° C. and 14 per cent moisture content of wheat.

At 35° C. and 17.4 per cent moisture content of the wheat it is possible that the concentration of carbon dioxide in the respiration chamber was high enough to depress the respiratory rate of the insects. Dendy and Elkington (1920) have shown that all *S. oryzae* placed in an atmosphere containing 23.2 per cent carbon dioxide become motionless in 18 to 43 hours, but on removal begin to creep around feebly. In some of their determinations on the carbon dioxide output of rice weevils they found the percentage of carbon dioxide to be about 10. They state: "Of course even this amount may have had some effect upon the respiratory exchange, but, for the short periods of time (24 to 48 hours) involved, the results suggest that this may be regarded as negligible." From some preliminary experiments carried on with this study, results indicated that after the concentration of carbon dioxide in the respiration chamber exceeded 10 per cent the carbon dioxide output tended to be depressed. These experiments were carried out on the granary weevil, only, and should be repeated for both rice and granary weevils. After converting the milligrams of carbon dioxide into cubic centimeters it was easy to calculate the percentage of carbon dioxide in their respiration chamber at the end of the experiments. At a temperature of 35° C. and a moisture content of the wheat of 17.4 per cent, the concentration of carbon dioxide in the chamber was slightly over 9 per cent, while at 35° C. and 15.2 per cent moisture content the concentration was slightly less than 6 per cent. Therefore, it is probable that at a concentration of 9 per cent the carbon dioxide was acting as a depressant; however, the lowered oxygen tension within the respiration chamber may also have been a contributing factor. Hargreaves, as quoted by Winterbottom (1922), states: "It seems that weevily wheat will use up the oxygen of the air in contact with the wheat and generate carbon dioxide, so that the resultant atmosphere will not support the life of the insect in wheat."

It is well known that as the temperature increases, the metabolism of the insect increases. Table 3 and Figure 3 show the effects of various temperatures on the carbon dioxide output of *S. oryzae*. Two sets of experiments were conducted, one in which the insects were placed in the respiration chamber without any food, and the other series in which the insects together with wheat with a moisture content of 14.6 per cent were placed in the chamber.

Table 3.—Milligrams of Carbon Dioxide Produced per Gram *S. oryzae* in 24 Hours at Various Temperatures

° C.	With wheat			Without wheat		
	Mgs. CO_2	Per cent loss in weight	Per cent dead	Mgs. CO_2	Per cent loss in weight	Per cent dead
10	13.8	.....	.....	13.6	3.0	0.0
15	45.5	.....	.....	19.6	7.4	0.0
20	110.4	.....	.....	36.3	8.7	.....
25	185.5	1.3	3.2	54.0	15.7	7.3
30	219.1	2.0	0.0	64.8	19.9	16.0
35	250.0	1.9	3.0	77.8	24.0	47.9
40	69.9	.....	100.0	46.5	41.9	100.0

It can be seen from Table 3 that in the presence of wheat very little loss of weight among the insects occurred at 35° C. or below, and the mortality was very low, but at 40° C. all the rice weevils were dead at the end of 48 hours. Without any wheat present there was much loss in weight at temperatures above 15° C., and at 30°, 35°, and 40° C. the mortality was high, increasing with each rise in temperature. The relation of carbon dioxide output to temperature is shown in Figure 3. The carbon dioxide output increases with each increase in temperature until 40° C. is reached, when a sudden decline in carbon dioxide production occurs. Unfavorable conditions and the resulting death of many

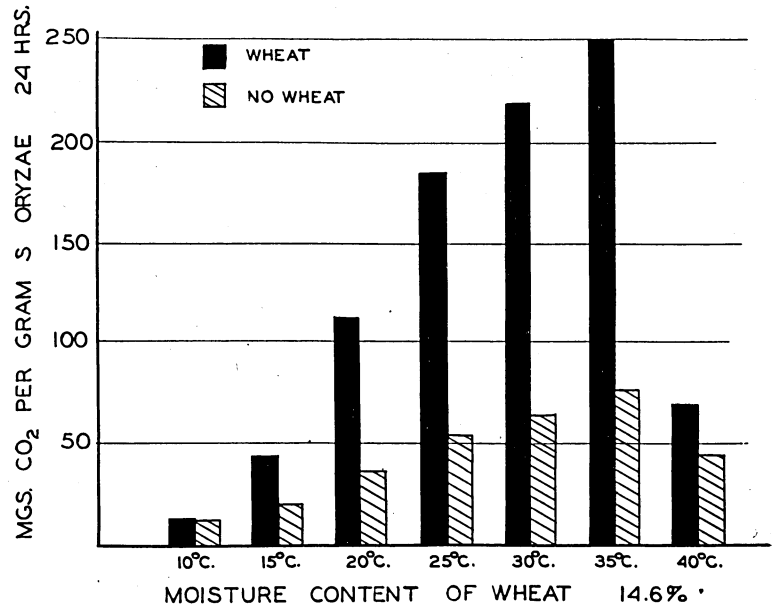

Fig. 3. Milligrams Carbon Dioxide Per Gram *S. oryzae* in 24 Hours at Various Temperatures

individuals causes this sharp falling-off. Barnes and Grove (1916) found that at 40° C. all rice weevils die within 38 hours in a moist atmosphere and 23 hours in a dry atmosphere. At 10° C. the carbon dioxide production was practically the same in the presence of food as in its absence, but at all the other temperatures worked with, the presence of food caused a great increase in the carbon dioxide output of the insect. This increase may be due to two reasons, the actual use of the food present, and the greater surface over which the insect can crawl.

Carbon Dioxide Production of *Sitophilus granarius* (the Granary Weevil)

The trend of the carbon dioxide production of the granary weevil is very similar to that of the rice weevil, with some slight differences, as can be seen from a comparison of the tables and graphs showing the carbon dioxide production of the granary weevil (Tables 4 and 5, Figures 4 and 5) with those of the rice weevil (Tables 2 and 3, Figures 2 and 3), the period of exposure being 48 hours. In Figure 4, the same results are shown as in Table 4 but divided by two to indicate the average for 24 hours.

Table 4.—Respiration of *S. granarius* in Wheat of Various Moisture Contents

Per cent moisture content of wheat	Per cent loss in weight of weevils		Per cent of dead weevils		Mgs. CO_2 per gram of weevils	
	25° C.	35° C.	25° C.	35° C.	25° C.	35° C.
8.7	14.8	29.7	0.5	48.8	238.6	229.0
10.7	4.1	13.7	0.0	5.6	334.8	425.8
14.0	0.0	2.2	1.4	0.0	339.4	489.8
15.2	2.5	1.8	0.4	0.0	341.6	496.4
17.4	0.2	0.4	0.4	1.4	381.6	450.2

Cole (1906) said that the rice weevil is distinctly less sensitive to desiccation than *S. granarius,* and that the former will live twice as long as the latter under the same condition of desiccation. Robinson (1926) found *S. oryzae* to be more sensitive to dryness in wheat than *S. granarius.* The present experimental results verify those of Robinson. Very little loss in weight or mortality of *S. granarius* occurred at 35° C. and 10.7 per cent moisture content of grain, while both the loss in weight and per cent dead were high for *S. oryzae;* neither did the carbon dioxide production of *S. granarius* drop off at 10.7 per cent as it did for *S. oryzae.* But at 8.7 per cent moisture content of wheat the loss in weight and mortality of *S. granarius* was high, and a decided reduction in the carbon dioxide output was obtained. As in the case of *S. oryzae,* the carbon dioxide production of *S. granarius* at 8.7 per cent was higher at 25° C. than at 35° C. (See Fig. 4.) At 35° C. and 17.4 per cent

moisture content of the wheat the carbon dioxide output of *S. granarius* was slightly depressed, owing probably, as with the rice weevil, to the relatively high concentration of carbon dioxide present (slightly more than 9 per cent) and the low oxygen tension.

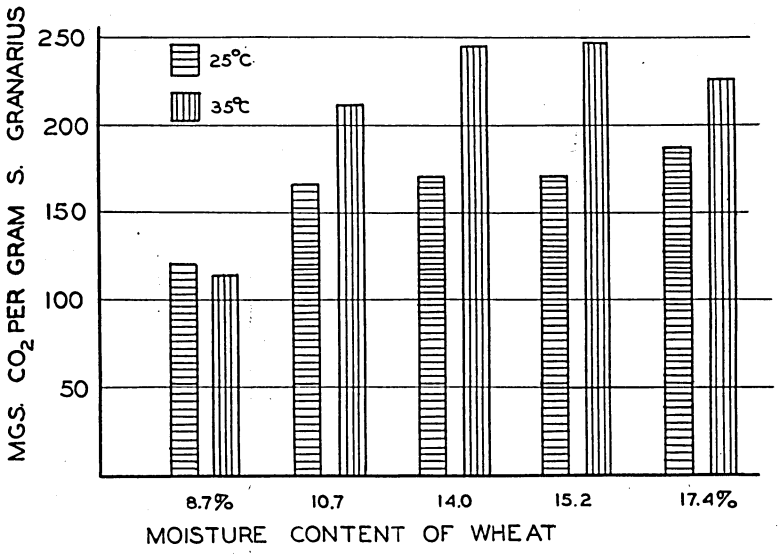

Fig. 4. Respiration of *S. granarius* in Wheat of Various Moisture Contents

When the moisture content of the wheat is 14 per cent or higher, the carbon dioxide output is greater for the rice weevil than for the granary weevil, weight for weight, but at a moisture content of 10.7 or 8.7 per cent the carbon dioxide output is greater for *S. granarius.* Dendy and Elkington (1920) state that "weight for weight, *S. granarius* at about 18°-19° C., gives off less carbon dioxide than *S. oryzae.*" They do not state the moisture content of the grain in which the insects were kept.

The effect of temperature on the rate of metabolism as measured by the carbon dioxide output of *S. granarius* is very similar to that of the rice weevil (compare Figures 3 and 5, and Tables 3 and 5). The wheat used in the experiments with *S. granarius* had the same moisture content as that used in the case of *S. oryzae,* i.e., 14.6 per cent.

At all temperatures except 40° C. the rice weevil has a higher carbon dioxide output than its close relative. At 40° C. it is perhaps the difference in the percentage dead that causes the reverse. It is interesting to note that in the presence of wheat the rice weevil produced more carbon dioxide at 30° C. than the granary weevil did at 35° C.

Table 5.—Milligrams of Carbon Dioxide Produced per Gram *S. granarius* in 24 Hours at Various Temperatures

° C.	With wheat			Without wheat		
	Mgs. CO_2	Per cent loss in weight	Per cent dead	Mgs. CO_2	Per cent loss in weight	Per cent dead
10	12.3	.....	.....	11.4	.....	.....
15	35.4	.....	.....	19.2	6.2	1.0
20	76.6	.....	.....	35.0	8.4	.....
25	155.8	2.1	1.8	45.9	9.7	.....
30	201.5	.....	.....	66.1	13.2	.....
35	218.8	1.1	1.4	78.1	13.0	1.8
40	138.3	.....	16.1	58.2	27.5	96.0

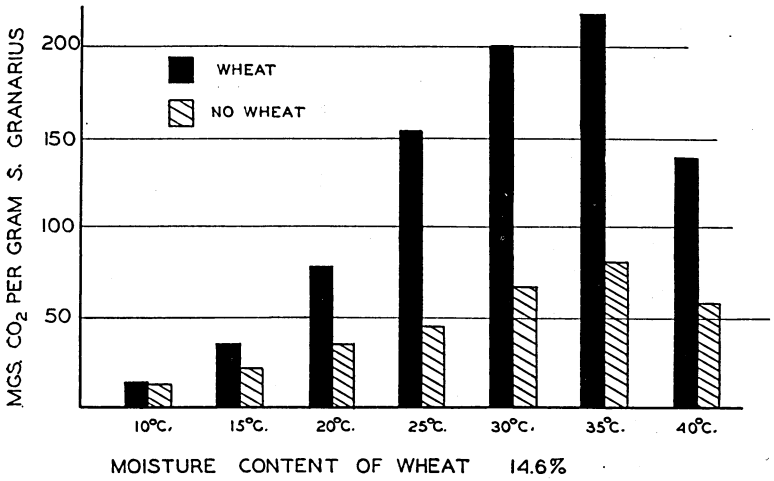

Fig. 5. Milligrams of Carbon Dioxide Per Gram *S. Granarius* in 24 Hours at Various Temperatures

Dendy and Elkington (1920), measuring the respiration of the rice and granary weevils, obtained results from 10 to 20 per cent lower than those obtained in this study. This difference may be accounted for by the smaller respiration chamber they used and the smaller amount of grain present for the insects to crawl around on. They used flasks with a volume of 150 cc. and only 50 grains of killed wheat, whereas the respiration chamber used in the present study had a volume of 950 cc. and 100 grams of wheat (dry basis) was present for the insects to crawl around on and eat.

Dendy and Elkington also found that 100 *S. oryzae* give off more carbon dioxide than 100 *S. granarius*. They state: "Experiments indicate that *S. granarius* gives off appreciably less carbon dioxide than *S. oryzae* even if we take into consideration only the number of weevils." This is not in accordance with the results of the experiments conducted

here, as can be seen from Table 6. These results indicate that the 70 granary weevils produced about the same amount of carbon dioxide as the 100 rice weevils.

Table 6.—Total Production of Carbon Dioxide in Milligrams by the Rice and Granary Weevils in 24 Hours

Per cent moisture content of wheat	Mgs. CO_2 produced			
	100 Rice weevils		70 Granary weevils	
	25° C.	35° C.	25° C.	35° C.
8.7	10.1	8.4	18.1	17.5
10.7	20.0	15.7	25.6	31.5
14.0	26.7	33.3	25.0	35.4
15.2	27.4	38.6	25.6	37.0
17.4	27.3	31.2	27.7	33.6

THE RELATION OF INSECT INFESTATION IN GRAIN TO HEAT AND MOISTURE PRODUCTION

Bailey (1921) states: "It is recognized that a determination of the respiratory rate does not afford a simple means of computing the temperature which the grain in question would attain under commercial conditions of storage. . . . The actual change of temperature depends not alone upon the rate of respiration of the grain but upon the size and shape of the bulk, the insulation afforded by the material of which the container or bin is constructed, and the temperature of the surrounding medium, and the initial temperature of the grain itself." The following discussion is a theoretical consideration of the part insects contribute to the process of heating of grain.

The reliability of the respiratory exchange as an index of heat production is variable and depends upon the nature of the metabolism. The substance catabolized can be determined within certain limits by means of the respiratory quotient, the relation by volume of the carbon dioxide output to the oxygen absorbed. The respiratory quotient for carbohydrates is 1, for fats .707, and for protein averages about .80. Even if the respiratory quotient is known, caution is necessary in its use. Incomplete oxidation may lower the respiratory quotient without change of the foodstuffs being burned. The storage of carbon dioxide as solid carbonate also may lower the respiratory quotient.

The respiratory quotient of the insects used in this study was not determined. Dendy and Elkington (1920) determined it for both *S. oryzae* and *S. granarius,* but their results varied a great deal. They state: "Our results as regards both species of weevil fall within the limits 0.97 and 0.72, as expressed by Krogh (1916)."

The calculations presented here on the production of heat and metabolic water by the weevil are based on the assumption that the

insects are burning carbohydrates only. There are several reasons for doing this: First, there are no data available as to what the weevils are utilizing, and with our present methods of attacking this problem, definite information would be difficult to obtain; second, the substrate upon which the weevils feed is chiefly carbohydrate, and, third, the calories of heat produced as calculated from the carbon dioxide output is lowest for carbohydrate combustion. That is, for every gram of carbon dioxide produced by oxidation of a carbohydrate, 2.56 calories of heat are liberated; of a fat, 3.37 calories are liberated, while a protein yields 2.84 calories.

By comparing Table 1 with Table 6 it can be seen that when the wheat has a moisture content of 15.2 per cent or less, the quantity of heat evolved as evidenced by the rate of respiration is much greater for the insects than for the wheat, but the rate for the wheat approaches that for the insects when the moisture content is as high as 17.4 per cent; that is, comparing 100 grams of wheat (dry basis) with 70 granary weevils (approximate weight 0.148 grams) or 100 rice weevils (approximate weight 0.136 grams). These infestations are not high as compared with the potential population under favorable conditions. It is interesting to note the effect of temperature on the rate of respiration of wheat with a moisture content of 17.4 per cent.

If carbohydrates are being oxidized by the insects, 2.56 calories are produced for each gram of carbon dioxide output, or each milligram of carbon dioxide is equivalent to 0.00256 calories. As the specific heat of wheat is 0.35 to 0.40 (Bailey), it would take 100 rice weevils or 70 granary weevils about five days at 25° C. to raise one gram of wheat one degree centigrade, or at 35° C. it would take them slightly more than four days. These figures may seem very small, but as compared with the amount of heat produced by wheat they are not insignificant. One gram of uninfested wheat at a moisture content of 15.2 per cent will require from 60 to 150 days to be raised one degree Centigrade, or at a moisture content of 17.4 per cent it will require from 7 to 11 days, depending on the temperature. Heating of grain at moisture contents which are not extreme is a long drawn out process, whereby these small units of heat tend to accumulate, the process becoming more and more rapid as the temperature increases. It is very probable that insects may initiate this process in wheat that contains 14 to 16 per cent water and greatly shorten the time that it takes wheat to reach dangerously high temperatures.

For each 2.9 grams of carbon dioxide produced by the insects there is one gram of water produced, assuming carbohydrates to be the substance oxidized, or for each milligram of carbon dioxide produced there

is 0.34 milligram of water liberated. At 25° C. it would take 70 granary weevils or 100 rice weevils about 100 days to raise the moisture content of 100 grams of wheat 1 per cent, or at 35° C. it would take them about 80 days. It would take wheat of 15.2 per cent moisture content from 15 to 30 times as long as this to produce the same amount of moisture. It seems that the production of this metabolic water must also be a very important factor in initiating the process of heating in wheat. Bailey has shown that above 14.78 per cent the rate of respiration is markedly increased. If insect-infested wheat is stored at a moisture content slightly lower than 14 per cent it is probable that the insects would raise it to above the critical point in a relatively short time.

In these calculations only the part the adult insects contribute has been considered, not taking into account the egg, larva, or pupa, and the ever-increasing population. The egg and pupa probably have a low respiratory rate, but that of the larva may be close to that of the adult.

CONCLUSIONS

Both the rice and the granary weevil have certain moisture requirements, but those of the granary weevil seem to be lower than those of the rice weevil. If there is insufficient moisture present the metabolic rate decreases, finally resulting in the death of the organism. If the moisture present is sufficient or more than sufficient (all other factors being constant), the metabolic rate remains fairly uniform.

Temperature has a decided effect on the rate of respiration of the species of weevils worked with. Of the temperatures used, the insects were the most active at 35° C. Progressing either way from this temperature the carbon dioxide production falls considerably.

If moisture conditions of the wheat are favorable, the rice weevil respires more than the granary weevil, weight for weight, but if individual weevils are compared, the granary weevil has the higher rate of respiration.

Grain infested with insects probably will tend to go "out of condition" much sooner than uninfested grain. The metabolic water and heat given off by the insects no doubt hasten this process and may even initiate it.

PART II. THE RELATION OF INSECT RESPIRATION TO THE TOXICITY OF FUMIGANTS

INTRODUCTION

It is generally believed that the susceptibility of an organism to a fumigant varies with the rate of respiration, and that, therefore, any factor which increases that rate automatically increases the efficiency of the fumigant. A rise in temperature and a moderate increase in the carbon dioxide content of the atmosphere surrounding the insect are two factors that increase the rate of respiration. It is known that the rate of metabolism varies with the stage of the insect's development, and that different species of insects may vary also in their respiratory rates. The present work was undertaken to determine whether the statement, "The susceptibility of an organism to a fumigant varies with the rate of respiration," would hold true for the various stages of certain stored-product insects.

HISTORICAL SUMMARY

Child (1915) states: "There can be no doubt that within a given species or organism a general relation exists between metabolic conditions and susceptibility to a given narcotic. Differences in susceptibility may be used with certain precautions and within certain limits as means of distinguishing the differences in metabolic conditions and more specifically in metabolic rate. Differences in metabolic condition do not exist independently of differences in condition of the colloid substratum and whether the narcotic affects primarily the substratum or certain of the chemical reactions, the susceptibility of the organism or part to its action must differ as the conditions which determine or are associated with metabolic activity differ."

Hazelhoff (1926) and (1928) observed that the width of the spiracle openings of insects is regulated normally by the amount of carbon dioxide pressure in the immediate neighborhood of the spiracles. He suggested that the penetration of hydrocyanic acid gas and other gases into the tracheal system might be accelerated and their insecticidal action thereby increased by the application of a small quantity of carbon dioxide.

Cotton (1932) concludes from experiments with fumigants on various stored-product insects that, "Other factors being normal, the susceptibility of an insect to a fumigant is influenced by any factor that affects the rate of metabolism of that insect. Any factor that increases the rate of metabolism increases the susceptibility of the insects to the action of a fumigant and vice versa.

"Of the known factors that increase the susceptibility of insects to fumigants, the three most important are: an increase in temperature; an increase in the carbon dioxide content of the fumigation chamber; and a decrease in the oxygen content of the fumigation chamber."

Cotton reported no original work on respiration of insects, but used the data of *(1)* Battelli and Stern (1913) on the respiration of various stages of the fly (not including the egg), *(2)* Helff (1927) who found that the rate of oxygen consumption in the larvae of five species of *Amblystoma* were widely divergent, and *(3)* Krogh (1916) showing the rate of metabolism of the pupa of the yellow mealworm (*Tenebrio molitor* L.) at different temperatures.

MATERIALS AND METHODS

The methods and materials used for the study of the respiration of the granary and rice weevil were similar to those described in Part I of this bulletin. An additional insect species was used, i.e., the confused flour beetle (*Tribolium confusum* Duv.), 70 adult insects, 70 pupae, or 60 larvae being used in each experiment, as it was found that these numbers were of almost equal weight (about 150 mgs.). The eggs used weighed 150.0 milligrams, but this was not an accurate measurement, as when the eggs are laid they are sticky, and much flour adheres to them. It is hard to say what percentage of the above weight is flour. In those experiments in which the adults and larvae were placed in flour, five grams of whole wheat flour was spread on the bottom of the respiration chamber.

In the fumigation experiments the methods of Strand (1930), with certain modifications as described by Lindgren and Shepard (1932), were followed. The insects to be fumigated were placed in bolting cloth cages and suspended two or three inches above the bottom of 6.2-liter Ehrlenmeyer flasks. The liquid fumigants were measured into the flasks with a pipette graduated to 0.01 cc. The flasks were then placed in a constant-temperature cabinet for five hours.

In all cases, except when otherwise stated, the figures given are in milligrams of fumigant per liter to give 50 per cent kill in five hours.

THE RATE OF RESPIRATION OF VARIOUS INSECT SPECIES AND THE SUSCEPTIBILITY TO FUMIGANTS

It is well known that the respiratory rate differs for species of insects in different genera. Even in the same genus the variations may be great. That toxicity of fumigants also varies with the species is equally well known among entomologists, and it is generally thought that toxicity is more or less dependent on the rate of respiration.

Table 7.—Carbon Dioxide Output of *T. confusum*, *S. oryzae*, and *S. granarius* and Their Susceptibility to Fumigants

	T. confusum adults	*S. oryzae* adults	*S. granarius* adults
Mgs. CO_2 per gm. insect in 24 hours at 25° C.	221.0	185.0	155.8
Mgs. carbon disulfide per liter to give 50% kill at 25° C.	63.0	24.0	40.0
Mgs. chloropicrin per liter to give 50% kill at 25° C.	4.4	2.0	5.6
Mgs. ethylene oxide per liter to give 50% kill at 25° C.	18.0	5.7	5.6

Table 7 and Figures 6 and 7 represent the relationship between the rate of respiration and toxicity of fumigants to insects of different genera and to two species of insects of the same genus. *S. oryzae,* weight for weight, respires more rapidly than does *S. granarius* and is more susceptible to carbon disulfide and chloropicrin but equal to *S. granarius* in its resistance to ethylene oxide.

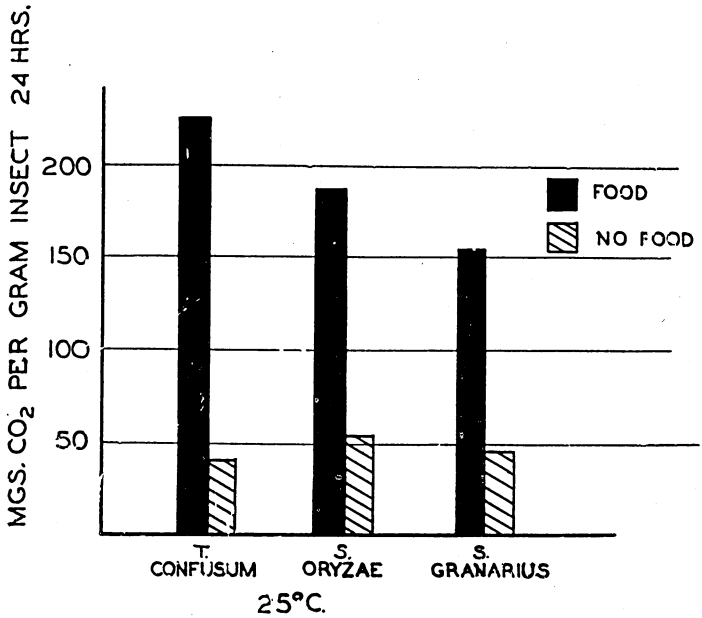

Fig. 6. Rate of Carbon Dioxide Output of *T. confusum, S. oryzae,* and *S. granarius*

T. confusum has the highest rate of respiration of the three insects tried, but requires a higher concentration of carbon disulfide and ethylene oxide to kill it. *S. granarius* is the most resistant to chloropicrin.

The relationship between toxicity and rate of respiration holds fairly well for *S. oryzae* and *S. granarius,* but there seems to be no correlation whatsoever between the two genera tried. Cotton (1932) found that it

required a 50 per cent higher dosage of carbon disulfide and ethylene oxide to kill the granary weevil than the rice weevil. These results were duplicated fairly well for both carbon disulfide and ethylene oxide when concentrations required to give 100 per cent kill are compared.

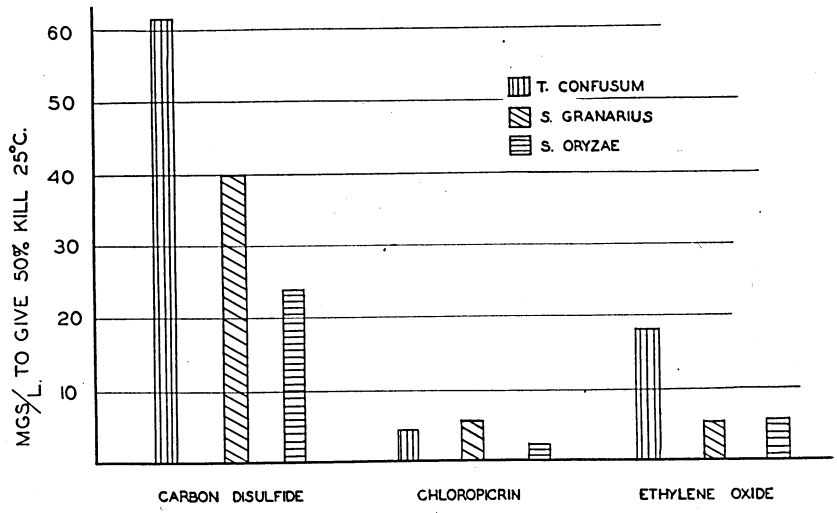

Fig. 7. Toxicity of Various Fumigants to *T. confusum, S. oryzae,* and *S. granarius*

RESPIRATORY RATES OF THE DIFFERENT STAGES OF *TRIBOLIUM CONFUSUM* AND THEIR SUSCEPTIBILITY TO FUMIGANTS

As there are marked differences in the metabolic rates of the various stages of an insect, a corresponding difference in susceptibility would be expected. From Table 8 and Figures 8, 9, 10, and 11 this does not always appear to be true.

Table 8.—Respiratory Rates of the Different Stages of the Confused Flour Beetle and the Susceptibility to Fumigants

		Egg	Larva	Pupa	Adult	
					Young	Old
Mgs. CO_2 per gm. insect in 24 hours at 25° C.	.....	11.9	163.1	19.3	40.1	221
Mgs. carbon disulfide per liter to give 50% kill at 25° C.	Low humidity	147	60	136	110	63
	High humidity	87	60	122	110	63
Mgs. chloropicrin per liter to give 50% kill at 25° C.	Low humidity	45	3.5	9.1	4.3	4.4
	High humidity	16	3.5	7.2	4.3	4.4
Mgs. ethylene oxide per liter to give 50% kill at 25° C.	Low humidity	2	11.0	19.5	.....	18
	High humidity	2	11.0	19.5	.....	18

Previous workers have found that the metabolic rate is greater during the embryonic stage than during metamorphosis. This may also be true with *Tribolium confusum,* but an exact weight of the eggs could not be obtained as so much flour adheres to them. The mortality figures for the eggs and pupae are average results obtained from groups of individuals of various ages, altho the egg and pupa vary as to their susceptibility to fumigants according to their age. The larvae were full grown, being ready to pupate within three or four days. The young adults were less than four days old, the older adults about six months old.

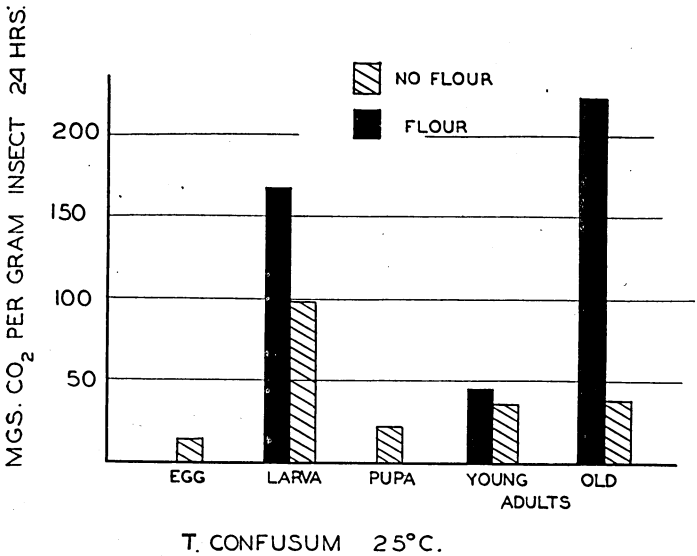

Fig. 8. Rate of Respiration of the Various Stages of *T. confusum*

The carbon dioxide output of old adults is very high in comparison to adults less than four days old (Fig. 8). The respiratory rate for young adults in flour is practically the same as for those without flour. It is probable that the young adult does not feed during the first few days.

The metabolic rate of both the egg and pupa is low in comparison to the larva or the adult. The carbon dioxide output of larvae, not in flour, is high, tending to show they have a large store of reserve food.

The larval stage is the most susceptible to carbon disulfide and chloropicrin, and second to the egg in susceptibility to ethylene oxide. The larvae have, next to the adults, the highest rate of respiration (Figs. 9, 10, and 11).

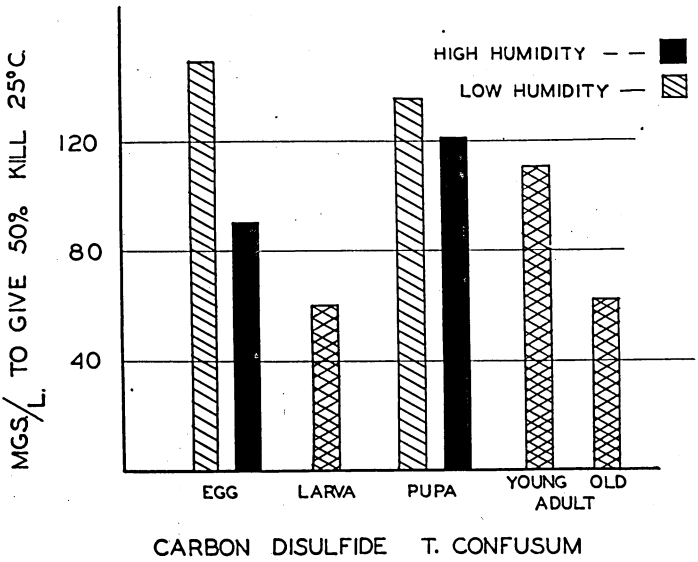

Fig. 9. Toxicity of Carbon Disulfide to the Various Stages of *T. confusum*

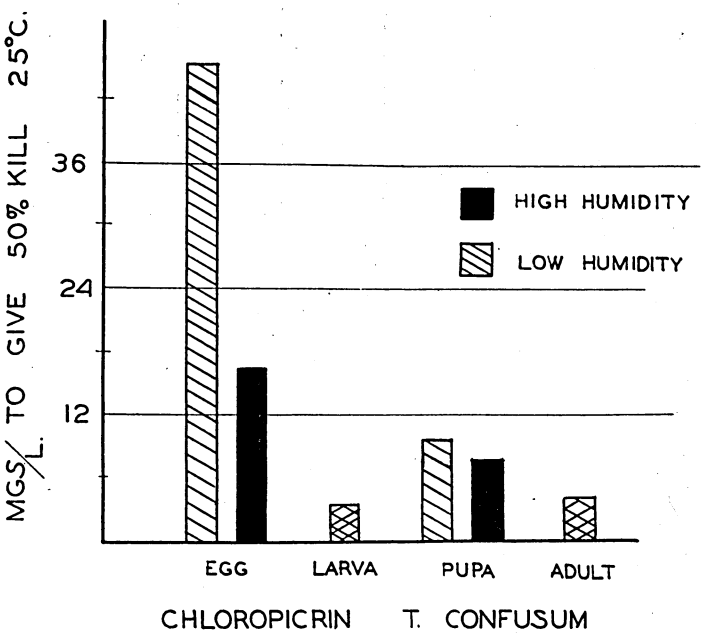

Fig. 10. Toxicity of Chloropicrin to the Various Stages of *T. confusum*

The pupae seem to be fairly stable in their resistance to the three fumigants tried. They are more resistant than either the adults or larvae to all three gases, and more resistant to ethylene oxide than any other stage.

The adults are in general more resistant to the fumigants than are the larvae, but have the highest rate of metabolism. Young adults have a much lower rate of respiration than old adults and these young individuals are harder to kill with carbon disulfide, but there is no difference in the susceptibility of the young and old adults to chloropicrin.

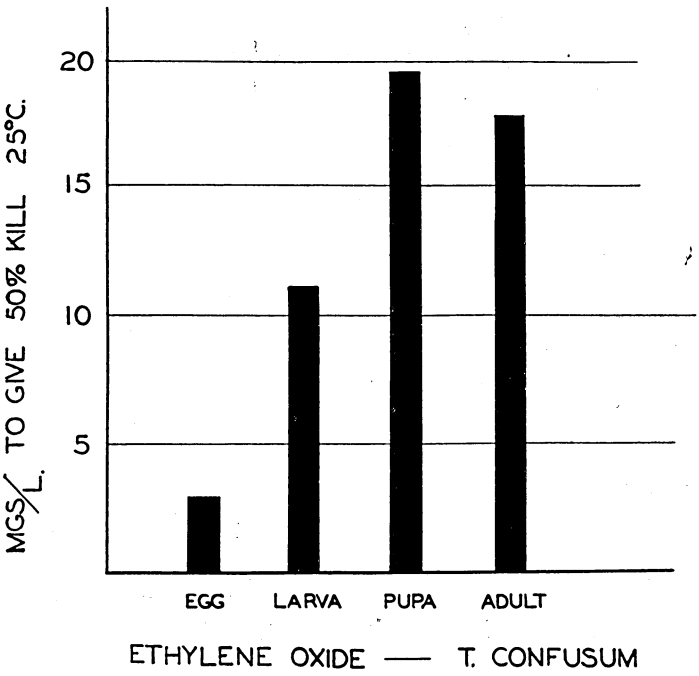

Fig. 11. Toxicity of Ethylene Oxide to the Various Stages of *T. confusum*

It is interesting to note that only the egg and pupal stages are affected by differences in the relative humidity at the time of fumigation. In all cases in which there are differences, a low humidity favors a greater resistance on the part of the insect.

Cotton (1932) concludes from his experiments with carbon disulfide on the confused flour beetle and the Mediterranean flour moth that the adults are the most susceptible, followed by the larvae and then the pupae. He did not work with the egg stage.

THE EFFECT OF TEMPERATURE ON THE RATE OF RESPIRATION OF *T. CONFUSUM* ADULTS AND ON THE SUSCEPTIBILITY TO FUMIGANTS

It is well known among entomologists that the rate of respiration of an insect increases with a rise in temperature within certain limits, and also the susceptibility of insects to fumigants increases with a rise in temperature. On the other hand, the previous paragraphs show that an insect species having a high respiratory rate is not necessarily more easily killed by a given fumigant than a species with a low rate.

Table 9.—The Effect of Temperature on the Rate of Respiration of *T. confusum* Adults and on the Susceptibility to Fumigants

	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.
Mgs. CO_2 per gm. insect in 24 hours at 25° C.	11.1	29.5	126.0	221.0	322.0	426.0
Mgs. carbon disulfide per liter to give 50% kill at 25° C. in 5 hrs.	154.0	86.0	76.0	63.0	44.0	32.0
Mgs. chloropicrin per liter to give 50% kill at 25° C. in 5 hrs.	11.5	7.1	5.9	4.4	2.8	1.8

This relationship of temperature to respiration and toxicity is well shown in Table 9 and Figures 12, 13, and 14. At 10° and 15° C. the carbon dioxide output is very low (Fig. 12), but a sudden increase is observed at 20° C. At a temperature of 17° C. or below, the confused flour beetle is very inactive. From 20° to 35° C. there is a uniform increase in the metabolic rate, but at 40° C. there is a sharp decline even tho no mortality took place. With no flour present at 40° C. there was

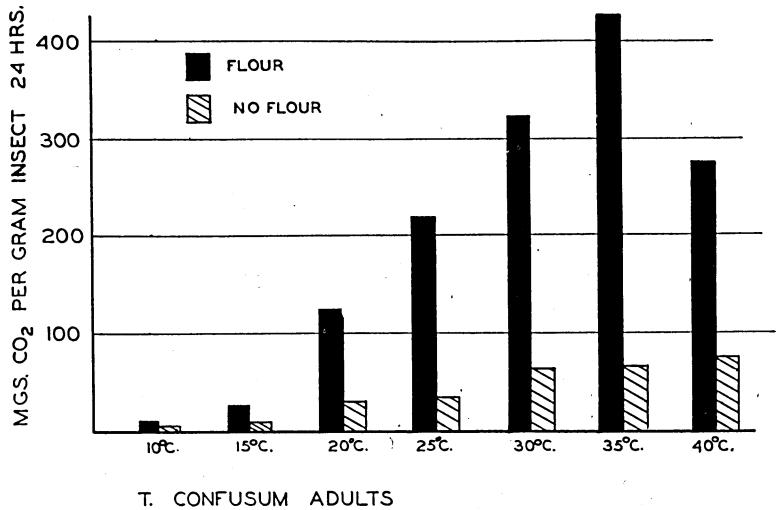

Fig. 12. Carbon Dioxide Output of *T. confusum* at Various Temperatures

an increase of 10 per cent in the carbon dioxide output over that at 35° C. There was no mortality at 35° C., whereas one per cent were dead at 40° C.

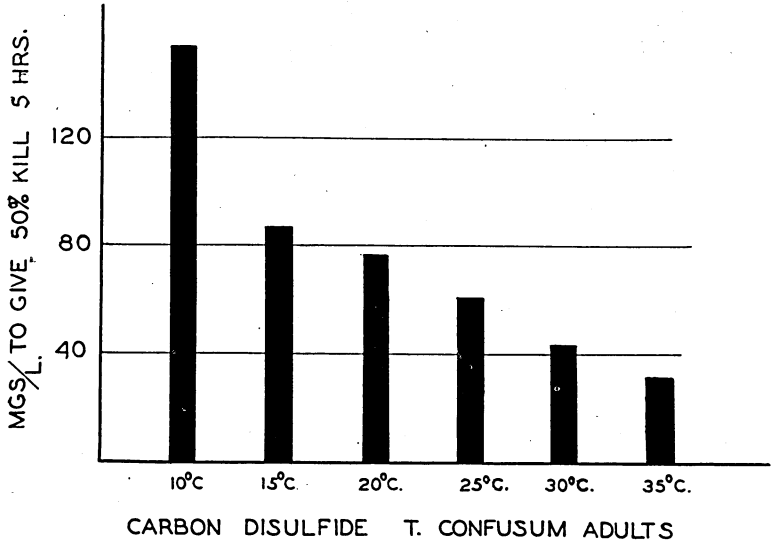

Fig. 13. Dosage of Carbon Disulfide to Give 50 Per Cent Kill of *T. confusum* Adults at Various Temperatures

Figures 13 and 14 show the increase in susceptibility of the confused flour beetle to carbon disulfide and chloropicrin as the temperature is increased. With both fumigants there is a sudden drop in resistance between 10° and 15° C., but from 15° to 35° C. the drop is gradual and regular. The decrease in resistance of the insect follows almost exactly the increase in the rate of respiration.

Battelli and Stern (1913) observed that the respiratory rate usually increases in the first few hours at a high temperature, and then regains a normal or falls to a lower level later. Cook (1927) found that after removal of *Chorizagrotis auxiliaris* from a cold room to a warm incubator, the rate of metabolism gradually increases for several hours, after which their metabolic rate gradually declines over two or three days to a lower constant figure. Cotton (1932) kept *Tribolium* at 50° F. and 80° F. for several days prior to fumigation. These insects were then fumigated for three hours at 80° F. He found it required almost twice the dosage of ethylene oxide to kill those insects kept at 50° F. prior to fumigation. Cotton states: "The initial difference in the metabolic rate is responsible for the great difference in quantity of fumigant needed." He apparently assumes that there is a depressive action on

the respiratory rate of insects removed from a low temperature to a higher one, whereas previous work as cited above tends to show the opposite.

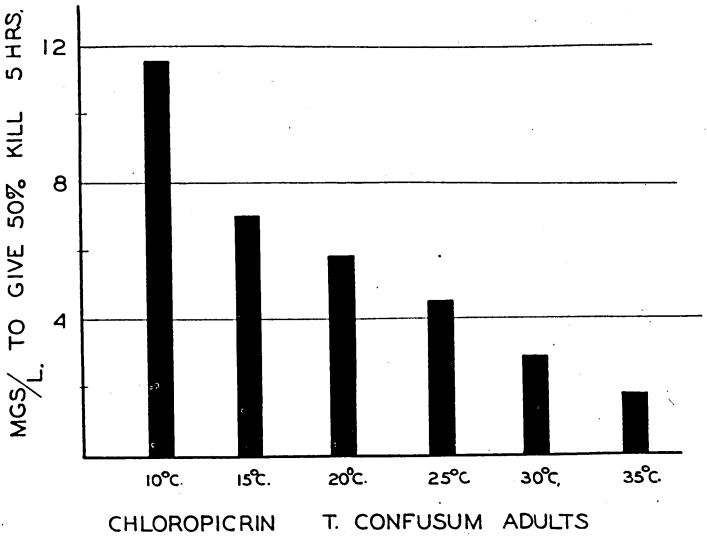

Fig. 14. Dosage of Chloropicrin to Give 50 Per Cent Kill of *T. confusum* Adults at Various Temperatures

THE RATE OF METABOLISM OF *T. CONFUSUM* PUPAE OF VARIOUS AGES AND THE SUSCEPTIBILITY TO FUMIGANTS

Investigation of the respiratory rate of pupae of various insects tends to show that the oxygen consumption and carbon dioxide output is high at first, then falls for some days and finally rises again, according to Sosnowski (1902), Weinland (1906), Tangl (1909), Krogh (1914), Taylor (1926), and Taylor and Steinback (1931). Similar results were obtained with *T. confusum* pupae. The first day the carbon dioxide output was high, followed the second day by a moderate decrease in the rate of respiration. From the second to the seventh day the carbon dioxide output was about constant, followed by a slight increase the eighth and ninth days.

Fumigation experiments were carried out to determine whether there was any difference in susceptibility of pupae of various ages. Figure 15 shows the results obtained using chloropicrin as the fumigant. A typical U curve is obtained, the pupae being least resistant at the beginning and at the end of the pupal stage, and most resistant from the second to the fourth day. A curve very similar to this was obtained for ethylene

oxide, but for carbon disulfide the dip in the curve occurred between the sixth and eighth days.

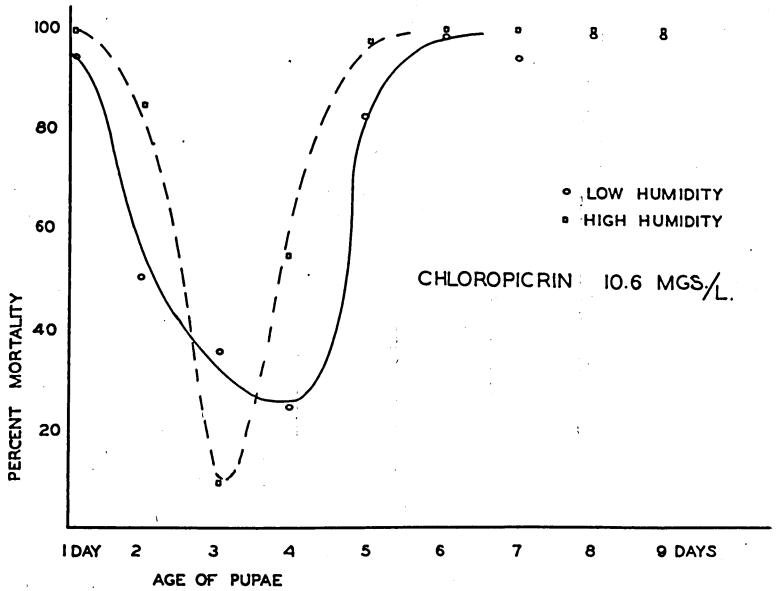

Fig. 15. Susceptibility to Chloropicrin of *T. confusum* Pupae of Various Ages

CONCLUSIONS

Comparative studies of the rate of respiration and the susceptibility to fumigants have been made of three species of stored-product insects. Altho *T. confusum* has the highest rate of respiration, it is the most resistant to carbon disulfide and ethylene oxide. The relationship between toxicity and rate of respiration holds fairly well for *S. oryzae* and *S. granarius*.

Studies similar to the above were carried out on the four stages of the confused flour beetle. From the data presented it seems there is no definite relationship between the rate of respiration of the different stages of an insect and their susceptibility to fumigants.

In comparing the susceptibility of insects or their stages to fumigants, the rate of respiration is not the only factor to take into consideration. Within a given stage any extrinsic factor that may tend to increase the rate of metabolism of that stage, may also tend to increase the susceptibility of it to fumigants. This is shown by the effect of temperature on both the rate of respiration and the susceptibility of adult insects to fumigants.

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